NOTES

—ON—

MICROSCOPICAL METHODS

FOR THE USE OF LABORATORY STUDENTS IN THE ANATOMICAL DEPARTMENT OF THE

CORNELL UNIVERSITY,

—BY—

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PREFATORY NOTE.

The following notes on microscopical methods take the place of those published in 1881. They are designed to accompany the Notes on Histological Methods printed last year, and to give only the main facts and principles relating to the microscope and to its manipulation which seem to the writer indispensable for the successful study of elementary histology.

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TOPICS.

The microscope and its parts—Care and use.—A microscope. A simple microscope. A compound microscope. Optical parts of a compound microscope. Mechanical parts (Fig. 3). Reflected light. Transmitted light. Central and oblique light. Lighting. Use of diaphragms and shading the object. Putting the objective in position and removing it. Function of the objective. Nomenclature of objectives. Putting the ocular in position and removing it. Function of an ocular. Nomenclature of oculars. Focusing. Working distance. Focusing with low objectives, with high objectives. Field of a microscope. Putting an object under the microscope. Care of a microscope,—the mechanical parts, the optical parts. Care of the eyes. (§§ 1–26, pp. 1–9; Figs. 1–4).

2. Interpretation of appearances under the microscope.—Dust or cloudiness on the ocular, on the objective. Relative position of objects or parts of the same object. Objects having irregular outlines. Transparent objects having curved outlines. Air bubbles. Oil globules. Distinctness of outline. Highly refractive. Doubly contoured. Optical section. Currents in liquids. Pedesis or Brownian movement. Demonstration of pedesis with the polarizing microscope. (§§ 27–45, pp. 9–14; Figs. 2, 4, 5).

3. Magnification—ocular micrometer ratio.—Magnification, expressed in diameters. Distance at which the image is measured.

Magnification of a simple microscope. Stage micrometer. Magnification of a compound microscope. Varying the magnification of a compound microscope. Ocular micrometer. Ocular micrometer ratio. Magnification of the objective, positive and negative oculars (note to § 57). Varying the ocular micrometer ratio. Table of magnifications and ocular micrometer ratios to be filled out by each student. (§§ 46–59, pp. 14–18; Figs. 1, 2, and 6).

4. **Micrometry and drawing.**—Micrometry with a simple microscope. Unit of measure in micrometry. Micrometry with a compound microscope, three methods. General remarks on micrometry. Drawing. Camera lucida. Camera lucidas that reflect the microscopic image—those that reflect the image of the drawing paper. Avoidance of distortion in using a camera lucida. Drawing with a camera lucida. (§§ 60–69, pp. 18–23; Figs. 6–8).

5. **Adjustable and immersion objectives, etc.**—Adjustable and non-adjustable objectives. Tube length. Dry and immersion objectives. Apochromatic objectives. Illuminators. (§§ 70–77, Figs. 9–11, pp. 24–29).

6. **Appendix.**—Imbedding in celloidin. Cutting, fixing and clearing celloidin sections. Counting white blood-corpuscles. Stronger cleaning mixture for glass. Cleaning large cover-glasses. Supplemental bibliography. (§§ 78–84, pp. 30–32).

THE MICROSCOPE AND ITS PARTS—CARE AND USE

(§ 1) **A microscope or magnifier** enables one to obtain an enlarged image of a near object.

(§ 2) **A simple microscope.**—With this an enlarged *erect* image of an object is seen (Fig. 1). This is easily demonstrated by placing a tripod magnifier on a printed page and holding the eye near the upper surface of the magnifier. A simple microscope is composed of one or more converging lenses.

(§ 3) **A compound microscope.**—This enables one to see an enlarged *inverted* image (Fig. 2).

(§ 4) **The optical parts of a compound microscope** always include the following: An *objective* or *object glass* (Figs. 2, 9, 10, 11), an *ocular* or *eye-piece* (Figs. 2, 11), and a *mirror* or some other apparatus for lighting the object under examination (Fig. 2–3).

For the mechanical parts see Fig. 3.

THE MIRROR—LIGHTING THE OBJECT.

(§ 5) **The mirror** should be freely movable, and have a plane and a concave face. The plane face is used for throwing a moderate amount of light on the object, or when it is necessary to have parallel rays or to know exactly the direction of the rays.

(§ 6) **Reflected, Incident or Direct Light** (Fig. 9).—It is by this kind of light that ordinary objects are seen; and objects so seen, whether with the microscope or the unaided eye, are said to be viewed as opaque objects.

(§ 7) **Transmitted light** (Fig. 10).—This, as the name implies, traverses the object instead of being reflected from its surface, as above (§ 6); hence an object to be examined by it must be approximately transparent. Objects seen by transmitted light are said to be viewed as transparent objects, or simply as transparencies. This is the kind of light usually employed with the compound microscope. Letters on thin paper may be seen by transmitted light if the paper is held between the eye and the source of light, and by reflected light if the paper is placed on the table.

(§ 8) **Axial or Central, and Oblique Light.**—Reference is here made to the *direction* of the rays which light an object, without regard to whether the light be reflected or transmitted.

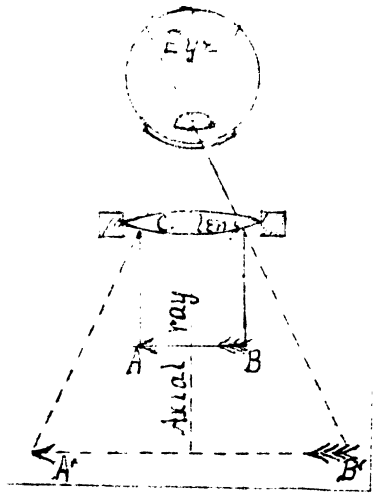

FIG. 1.—Diagram of the simplest form of a simple microscope.

A–B.—The object. This is placed between the simple microscope and its principal focal point. The *eye* of the observer is on the opposite side.

A′–B′—The virtual image of the object as seen by the eye. It is erect and on the same side of the magnifier as the object.

Axial ray.—Central or axial ray of the image.

C. Lens.—Converging lens. A simple microscope always consists of one or more converging lenses.

Light is said to be axial or central either when the object is lighted with rays which are parallel to the optic axis of the microscope, as with the plane mirror (Fig. 10), or when the central or axial ray from the concave mirror is parallel to that axis.

Light is said to be oblique either when an object is lighted by parallel rays which make an angle with the optic axis of the microscope (Figs. 9, 10), or when the central or axial ray from the concave mirror makes an angle with that axis.

(§ 9) **Lighting.**—Proper lighting is one of the essentials in microscopy. In general, the beginner should try all methods of lighting the object under examination. It is only in this way that one can learn to light properly and quickly.

Unmodified sunlight should not be employed. North light is best and most uniform. It should come from the left. If artificial light must be employed, use a lamp that does not flicker.

Learn first to throw the light in the desired direction, viz.: up through the aperture in the stage or down through it. To do this, place a piece of printed paper over the aperture in the stage; grasp the mounting of the mirror and turn it until the paper is lighted. Swing the mirror above the stage and throw the light upon the paper over the aperture. Use first the plane and then the concave mirror.

(§ 10) **Use of diaphragms.**—In order to get the best effect, only such a part of the object should be lighted as can be brought into the field of view at once. To attain this, an opaque perforated screen—called a diaphragm—is placed between the mirror and the object. The opening or perforation in the diaphragm selected should be of about the same size as the front lens of the objective to be employed.

The diaphragm should be very close to the object when high objectives are used, or fine details are to be made out.

Shading the object.—To get the clearest image of an object no light should reach the eye except from the object. A handkerchief or a dark cloth wound around the objective will serve the purpose. Often the proper effect may be obtained by simply shading the top of the stage with the hand or with a piece of bristol-board. [(**Bı**, 22), (**Bau.** 42, 52), (**Beh.** 82), (**Bz**, 5), (**C.** 155, 169), (**Car.** 54), (**Fol.** 38), (**Frey** 22, 84), (**H.** 162), (**Le Conte**), (**N. & S.** 92), (**Robin** 52, 329)].

THE OBJECTIVE

(§ 11) **Putting the objective in position and removing it.**—Elevate the body of the microscope by means of the coarse adjustment (Fig. 3), so that there may be plenty of room between its lower end and the stage. Grasp the objective lightly near its lower end with two fingers of the left hand, and hold it against the nut in the lower end of the body (Fig. 3). With two fingers of the right hand take hold of the

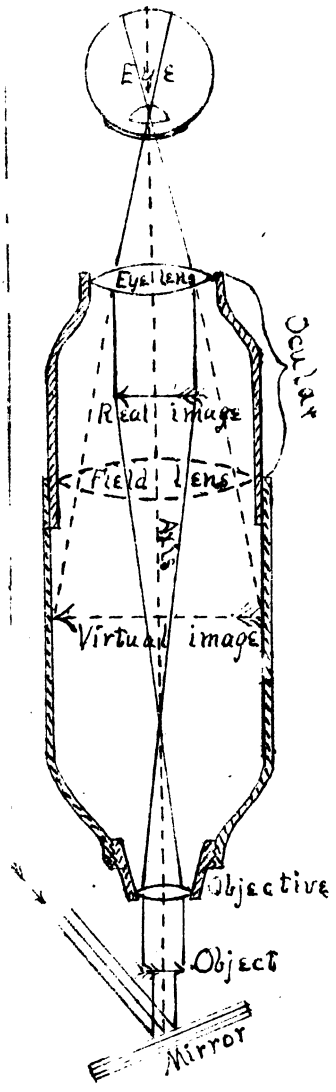

FIG. 2.—Diagram of the optical parts of a compound microscope, showing their relation and function.

Axis.—The optical axis of the microscope. The axial ray of the image takes this path.

Eye.—The eye of the observer.

Eye Lens.—The upper lens of an ocular,—the one next the eye of the observer.

Field Lens.—The lower lens of the ocular.

Mirror.—This is for reflecting light upon the object.

Object.—The object to be examined.

Objective.—The optical part of the microscope next above the object, and forming the real image.

Ocular.—The optical part next the eye.

Real image.—This is the image formed by the objective. It is called real because it actually exists independently of the eye. It is the same kind of an image as that seen in a photographer's camera.

Virtual image.—This is the image which the eye sees when looking into the microscope. It is an image of the real image. Compare with Fig. 1.

milled ring near the back or upper end of the objective, and screw it into the body of the microscope. Reverse this operation for removing the objective. By following this method the danger of dropping the objective will be avoided.

(§ 12) **Demonstration of the function of the objective.**—Put a two-inch objective, or the back combination of the three-fourths inch in position. Place some printed letters or figures under the microscope, and light well. In place of an ocular, put a piece of ground glass over the upper end of the body of the microscope.

Lower the body by means of the coarse adjustment (Fig. 3), until the objective is within 2–3 cm. of the object on the stage. Look at the ground glass on the top of the body, holding the head about as far from it as for ordinary reading, and slowly elevate the body by means of the coarse adjustment until the image of the letters appears on the ground glass.

The image can be more clearly seen if the object is in a strong light and the ground glass in a moderate light.

The letters will appear as if printed on the ground glass, but will be inverted (Fig. 2).

If the objective is not raised sufficiently, and the head is held too near the ground glass, the objective will act as a simple microscope. If the letters are erect, and appear to be down in the microscope and not on the ground glass, hold the head farther from the ground glass, shade the latter, and raise the body of the microscope until the letters do appear on the ground glass.

The function of an objective, as seen from this experiment, is to form an enlarged, inverted, real image of an object, this image being formed on the opposite side of the objective from the object (Fig. 2).

(§ 13) **Nomenclature of Objectives.**—The Continental opticians number the objectives, 1, 2, 3, etc., No. 1 being lowest. Letters are also sometimes used, those farther along in the alphabet being usually applied to the higher powers. American and English opticians mark them with their equivalent focal length. In this method, the smaller the number, whether whole or fractional, the greater the power. For example, an objective marked 6 mm. or 1-4 in. signifies that, at the standard distance (25 cm. or 10 in.), it would give an image of approximately the same size as a simple converging lens whose principal focal distance is 6 mm. or 1-4 in. This image would be larger than that formed by an objective marked 12 mm. or 1-2 in., and smaller than that formed by one marked 3 mm. or 1-8 in., etc. Comparatively objectives are said to be high or low as they magnify much or little. Other things being equal, the general appearance of a high objective is more complex than a low one ; and the front glass is smaller in a

high than in a low objective.* [(Bi, 6, 8), (Bau. 17), (Beh. 22), (C. 9, 13, 192–195), (Frey 11), (H. 16). (See also references under adjustable and immersion objectives near the end)].

THE OCULAR.

(§ 14) **Putting the ocular in position and removing it.**—Elevate the body of the microscope with the coarse adjustment (Fig. 3), so that the objective will be 2 cm. or more from the object—grasp the ocular by the milled ring next the eye lens (Fig. 2, 11), and the coarse adjustment or the tube of the microscope and gently force the ocular into position. In removing the ocular reverse the operation. If the above precautions are not taken, and the oculars fit snugly, there is danger in inserting them of forcing the body of the microscope downward and the objective upon the object.

(§ 15) **Function of an ocular.**—Look at the real image on the ground glass (§ 12), with a simple microscope. The image seen with the simple microscope is merely an enlargement of the one on the ground glass,—the letters still remaining inverted.

Remove the ground glass, and put the **A** ocular in position. Hold the head so that one eye will be close to the eye lens. The image of the letters will appear as when using the simple microscope (§ 2), but the image will become more distinct by raising the body of the microscope slightly with the coarse adjustment.

The function of the Ocular, as seen from the above, is that of a simple microscope, viz.: It magnifies the real image formed by the objective precisely as if that image were an object. Compare the image formed by the ocular (Fig. 2), and that formed by a simple microscope (Fig. 1).

The *field lens* of the ocular (Fig. 2), makes the image smaller, and at the same time brighter. One may demonstrate this by removing the field lens. (See also note under § 57).

(§ 16) **Nomenclature of Oculars.**—The Continental opticians number the oculars, 1, 2, 3, etc., no. 1 magnifying least.

American and English opticians generally mark them with letters, **A, B, C,** etc., **A** being lowest.

American and English opticians also mark the oculars with their equivalent focal length as for objectives. The power increases as the focal length diminishes, as with objectives.

Oculars are said to be high or deep when they magnify greatly, and low or shallow when they magnify little. Other things being equal, the longer an ocular the lower it is in magnification, and con-

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*The student is referred to (§ 46–54) for a consideration of magnifying power or magnification.

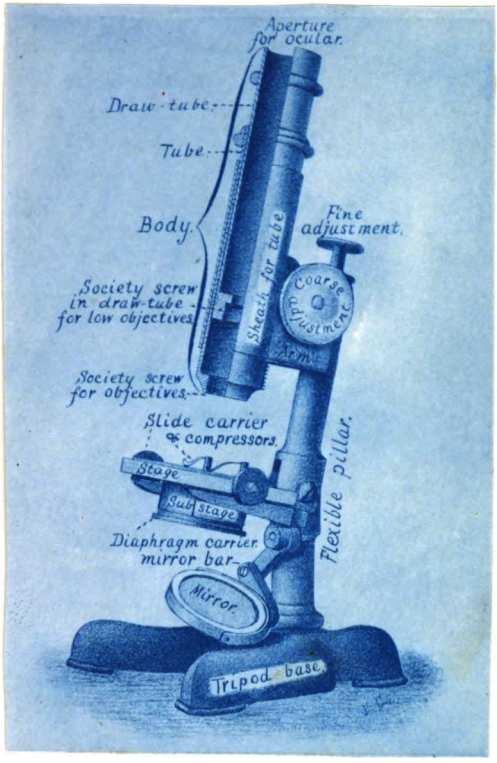

FIG. 3.—Stand of a Laboratory Compound Microscope with the parts named. A section has been removed from the body to show the position of the screw for the objective in the lower end of the body, and of the draw-tube.

versely the shorter it is the higher is its magnification. So also the larger the eye lens the lower is the ocular and the smaller the eye lens the higher.* [(**Bi**, 7, 30, 42), (**Bau.** 34), (**Beh.** 35–38), (**C.** 27, 109), (**N. & S.** 28)].

FOCUSING.

(§ 17) Focusing is arranging the object and the microscope in such a way that a clear image may be seen.

With the simple microscope, either the object or the microscope may be moved; but with the compound microscope the object more conveniently remains stationary on the stage and the body of the microscope is changed in position.

In general, the higher the power of the microscope, the nearer together must the object and the microscope be brought.

With the compound microscope, the higher the objective or ocular,† or the longer the body of the microscope, the nearer must the objective be brought to the object.

(§ 18) **Working distance.**—By this is meant the space between the front of the objective and the object when the microscope is in focus. This distance is always *less* than the nominal focal length of the objective. For example, the front or lower part of a 6 mm. or 1-4 inch objective would not be 6 mm. or 1-4 inch from an object when it was in focus, but considerably *less* than that.

(§ 19) **Focusing with low objectives.**—Place a mounted fly's wing under the microscope (§ 22); put the three-fourths objective in position (§ 11), and also the lowest ocular. Select the proper opening in the diaphragm (§ 10), and light the object well with transmitted light (§ 9).

Hold the head at about the level of the stage, look toward the window, and between the object and the front of the objective; with the coarse adjustment lower the body (Fig. 3), until the objective is within about half a cm. of the object. Then look into the microscope and slowly elevate the body with the coarse adjustment. The image will appear dimly at first, but will become very distinct by turning the body still higher. If the body is raised too high the image will become indistinct, and finally disappear. It will again appear if the body is lowered the proper distance.

When the instrument is well focused, change the position of the mirror and note the effect. Swing the mirror above the stage and

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*The student is referred to § 46–54 for a consideration of magnifying power or magnification.

† Some microscope makers so arrange the oculars that they enter the tube of the microscope the proper distances, so that it is not necessary to refocus after changing oculars (**M. B.**, 1886, pp. 9, 31).

throw the light upon the object instead of up through it, then swing the mirror back beneath the stage.

Put the **C** ocular in place of the **A**. It will be found necessary to lower the body somewhat to get the instrument in focus.

Pull out the draw tube 4–5 cm., thus lengthening the body of the microscope, and it will be found necessary to lower the body still more (§ 17).

(§ 20) **Focusing with high objectives.**—Employ the same object as before, elevate the body of the microscope and remove the three-fourths objective as directed. Put the 1–5 or a higher objective in place, and exchange the C ocular for the A, and push in the draw-tube.

To push in the draw-tube, grasp the large milled ring of the ocular with one hand, and the milled head of the coarse adjustment with the other. If this were done without these precautions the objective might be forced against the object and the ocular thrown out by the compressed air.

Light well, and employ the proper opening in the diaphragm, etc. (§9, 10). Look between the front of the objective and the object as before (§ 19), and lower the body with the coarse adjustment till the objective almost touches the cover-glass over the object. Look into the microscope, and, with the coarse adjustment, raise the body very slowly until the image begins to appear, then turn the milled head of the fine adjustment (Fig. 3), first one way and then the other, if necessary, until the image is sharply defined.

Note that this high objective must be brought nearer the object than the low one, and that by changing to a higher ocular or lengthening the body it will be found necessary to bring the objective still nearer the object, as with the low objective (§ 19).

Always focus up, as directed above. If one lowers the body only when looking at the end of the objective as directed above, there will be no danger of bringing the objective in contact with the object, as may be done if one looks into the microscope and focuses down.

When the instrument is well focused, move the object around in order to bring different parts into the field of view (§ 21). It may be necessary to re-focus with the fine adjustment every time a different part is brought into the field. In practical work, one hand is kept on the fine adjustment constantly, and the focus is continually varied.

(§ 21) **Field of a microscope.**—The field or field of view of a microscope, is the part of an object or the space visible at one view. If the object or objective is shifted, a different field is brought into view. If the mirror is properly arranged, and there is no object under the microscope, the field appears as a circle of light.

The size of the field of a microscope may be exactly determined by

placing a stage micrometer (§ 52), under the microscope and counting the spaces in the field.

(§ 22) **Putting an object under the microscope.**—This is so placing an object under the simple microscope, or on the stage of the compound microscope, that it will be in the field of view when the microscope is in focus (§ 21).

With low powers, it is not difficult to get an object under the microscope. The difficulty increases, however, with the power of the microscope and the smallness of the object. It is usually necessary to move the object in various directions while looking into the microscope, in order to get it into the field. Time is usually saved by getting the object in the center of the field with a low objective before putting the high objective in position. This is greatly facilitated by using a double nose-piece, or revolver.*

CARE OF THE MICROSCOPE.

(§ 23) The microscope should be handled carefully, and kept perfectly clean. The oculars and objectives should never be allowed to fall.

When not in use keep it in a place as free as possible from dust.

All parts of the microscope should be kept entirely free from liquids, especially from acids, alkalies, alcohol, benzine, turpentine and chloroform.

(§ 24) **Care of the mechanical parts.**—To clean the mechanical parts put a small quantity of fine sewing-machine oil on a piece of clean chamois or soft cloth and rub the parts well. Remove the oil by wiping with a clean piece of cloth or chamois. If the mechanical parts are kept clean in this way, no lubricator is necessary.

In inclining the microscope, grasp some part or parts that will not bring any strain on the fine adjustment.

(§ 25) **Care of the optical parts.**—These must be kept scrupulously clean in order that the best results may be obtained.

Glass surfaces should never be touched with the fingers, for that will soil them.

The glass of which the lenses are made is quite soft, consequently

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*As specimens are sometimes very small, or some part of a large specimen shows a particular structure with special excellence, it is desirable to so mark the preparation that the minute object or the part of a large object may be found quickly and with certainty. A simple way to do this is to find the object under the microscope, and then place a minute spot of Brunswick black at one side. After this is done, remove the slide from the stage and surround the object with a ring of Brunswick black, making the ring as small as possible and not cover the object. It will then always be known that the part to be examined is within the ring (B. 1, 47, C. 117).

it is necessary that only soft, clean cloths or paper be used in wiping them.

"*Paper for cleaning the lenses of objectives and oculars.*—For the last two years the so-called Japanese filter paper (the bibulous paper often used by dentists when filling teeth), has been used in the laboratory for cleaning the lenses of oculars and objectives, and especially for removing the fluid used with immersion objectives. Whenever a piece is used once it is thrown away. It has proved more satisfactory than cloth or chamois, because dust and sand are not present; and from its bibulous character it is very efficient in removing liquid or semi-liquid substances." [(**M.** 1886, p. 265)].

Dust may be removed with a camel's hair brush.

Cloudiness may be removed from the glass surfaces by breathing on them, then wiping quickly with a soft cloth or the bibulous paper.

Cloudiness on the inner surfaces of the ocular lenses may be removed by unscrewing them and wiping as directed above. A high objective should never be taken apart by an inexperienced person.

If the cloudiness cannot be removed as directed above, moisten one corner of the cloth or paper with 95 per cent. alcohol, wipe the glass first with this, then with the dry cloth or the paper.

Water may be removed with soft cloth or the paper.

Glycerin may be removed with cloth or paper saturated with distilled water; remove the water as above.

Blood or other albuminous material may be removed while fresh with a moist cloth or paper the same as glycerin. If the material has dried to the glass, it may be removed more readily by adding a small quantity of ammonia to the water in which the cloth is moistened, (water 100 cc., ammonia 1 cc).

Canada Balsam, damar, paraffine, or any oily substance, may be removed with a cloth or paper wet with chloroform, turpentine or benzine. The application of these liquids and their removal with a soft, dry cloth or paper should be as rapid as possible, so that none of the liquid will have time to soften the setting of the lenses.

Shellac Cement may be removed by the paper or a cloth moistened in 95 per cent. alcohol.

Brunswick Black, *Gold Size*, and all other substances soluble in chloroform, etc., may be removed as directed for balsam and damar. [(**Bi**, 238), (**Bau.** 85), (**Beh.** 96), (**C.** 159), (**Frey** 91), (**R.** 9), (**J. N. Y. m. s.** 1885, p. 113)].

CARE OF THE EYES.

(§ 26) Keep both eyes open, using the eye-screen if necessary (Fig. 4); and divide the labor between the two eyes, i. e., use one eye for observing the image awhile and then the other. In the beginning it is not advisable to look into the microscope continuously for more

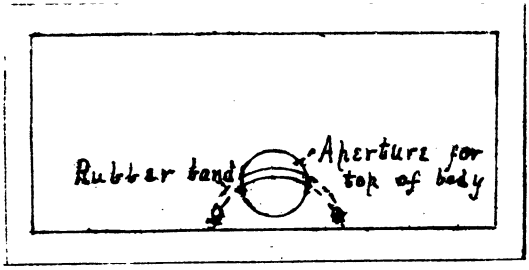

FIG. 4.—*Eye Screen.*—The eye screen is formed by pasting black velveteen to the upper surface of a bristol-board 10x25 cm. A hole large enough to receive the body of the microscope is cut at the middle of the length and nearer one side, as shown in the figure. A rubber band is tied through the small holes indicated in the figure, thus forming a loop. To put the screen in position, this loop is put around the top of the body, then the latter is put through the hole in the screen. Finally the ocular is put in place.

than half an hour at a time. One never should work with the microscope after the eyes feel fatigued. After one becomes accustomed to microscopic observation he can work for several hours with the microscope without fatiguing the eyes. This is due to the fact that the eyes become inured to labor like the other organs of the body by judicious exercise. It is also due to the fact that but very slight accommodation is required of the eyes; the eyes remaining nearly in a condition of rest as for distant objects. The fatigue incident upon using the microscope at first is due partly at least to the constant effort to accommodate the eye for a near object. With a microscope of the best quality, and suitable light—that is light which is steady and not so bright as to dazzle the eyes nor so dim as to strain them in determining details—microscopic work should improve rather than injure the sight. [(C. VII–VIII, 158), (J. r. m. s. 1886, p. 700), (Z. w. M. 1884, p. 20)].

INTERPRETATION OF APPEARANCES UNDER THE MICROSCOPE.

The following sections on interpretation are meant to give the student a few examples of the appearances constantly arising in microscopic work, so that they may not puzzle him nor lead him into error. The exercises also furnish an excellent drill in manipulation.

(§ 27) **Dust or cloudiness on the Ocular.**—Employ the 3-4 objective, A ocular, and fly's wing, as object.

Unscrew the field-lens and put some particles of lint or dark cloth on its upper surface. Replace the field-lens and put the ocular in position (§ 14). Light the field well and focus sharply. The image will be clear, but part of the field will be obscured by the irregular outline of the particles of lint. Move the object to make sure this appearance is not due to it.

Grasp the ocular by the milled ring, just above the body of the microscope, and rotate it. The irregular object will rotate with the ocular. Cloudiness or particles of dust on any part of the ocular, may be detected in this way.

(§ 28) **Dust or cloudiness on the Objective.**—Employ the same ocular and objective as before (§ 27), and the fly's wing as object. Focus and light well, and observe carefully the appearance. Rub glycerin on one side of a slide near the end. Hold the clean side of this end close against the objective. The image will be obscured, and cannot be made clear by focusing. Then use a clean slide, and the image may be made clear by elevating the body slightly. The obscurity

produced in this way is like that caused by clouding the front glas of the objective. Dust would make a dark patch on the image that would remain stationary while the object or ocular was moved.

If too small a diaphragm is employed, only the central part of the field will be illuminated, and around the small light circle will be seen a dark ring.

(§29) **Relative position of objects or parts of the same object.**—The general rule is that objects highest up come into focus *last* in focusing up, *first* in focusing down.

(§ 30) **Objects having plane or irregular outlines.**—As object use three printed letters mounted in canada balsam (Hist. Notes, § 27a, p. 17). The first letter is placed directly upon the slide, and covered with a small piece of glass about as thick as a slide. The second letter is placed upon this and covered in like manner. The third letter is placed upon the second thick cover and covered with an ordinary cover glass. The letters should be as near together as possible, but not overlapping. Employ the same ocular and objective as above (§ 27).

Lower the body till the objective almost touches the top letter, then look into the microscope, and focus slowly up. The lowest letter will first appear, and then, as it disappears, the middle one will appear, and so on. Focus down, and the top letter will first appear, then the middle one, etc. The relative position of objects is determined exactly in this way in practical work.

(§ 31) **Transparent objects having curved outlines.**—The success of these experiments will depend entirely upon the care and skill used in preparing the objects, in lighting, and in focusing.

Employ a 1-5 or higher objective and the **C** ocular for all the experiments. It may be necessary to shade the object (§ 10) to get satisfactory results. When a diaphragm is used the aperture should be small (§ 10).

(§ 32) **Air bubbles.**—Prepare these by placing a drop of mucilage on the centre of a slide and beating it with a scalpel blade until the mucilage looks milky from the inclusion of air bubbles. Put on a cover-glass (Hist. notes, § 3, 6), but do not press it down.

(§ 33) **Air bubbles with central illumination.**—Shade the object; and with the plane mirror, light the field with central light (Fig. 10, **a**, **b**).

Search the preparation until an air bubble is found appearing about 1 mm. in diameter. If the light is central the air bubble will appear with a wide dark circular margin and a small bright center. If the bright spot is not in the center, adjust the mirror until it is.*

* This is one of the simplest and surest methods of telling when the light is central or axial.

Focus both up and down, noting that in focusing up the central spot becomes very clear and the black ring very sharp. On elevating the body still more the center becomes dim, and the whole bubble loses its sharpness of outline.

(§ 34) **Air bubbles with oblique illumination.**—Remove the substage of the microscope (Fig. 3), and all the diaphragms. Swing the mirror so that the rays may be sent very obliquely upon the object (Fig. 10, **c**). The bright spot will appear no longer in the center but on the side *away from* the mirror (Fig. 5, **A**).

(§ 35) **Oil globules.**—Prepare these by beating a small drop of clove oil with mucilage on a slide and covering as directed for air bubbles (§ 32).

(§ 36) **Oil globules with central illumination.**—Use the same diaphragm and light as above (§ 33). Find an oil globule appearing about 1 mm. in diameter. If the light is central the bright spot will appear in the center as with air (§ 33). Focus up and down as with air; and note that the bright center of the oil globule is clearest *last* in focusing up.

(§ 37) **Oil globules with oblique illumination.**—Remove the substage, etc., as above, and swing the mirror to one side and light with oblique light. The bright spot will be eccentric, and will appear to be on the *same* side as the mirror (Fig. 5, **B**).

(§ 38) **Oil and air together.**—Make a preparation exactly as described for air bubbles (§ 32), and add at one edge a little of the mixture of oil and mucilage (§ 35); cover and examine.

The substage need not be used in this experiment. Search the preparation until an air bubble and an oil globule, each about 1 mm. in diameter, are found in the same field of view. Light first with central light, and note that in focusing up the air bubble comes into focus first and that the central spot is smaller than that of the oil globule. Then, of course, the black ring will be wider in the air bubble than in the oil globule. Make the light oblique. The bright spot in the air bubble will move *away from* the mirror while that in the oil globule will move *toward* it. Compare figure 5 A and B.*

(§ 39) **Air and oil by reflected light.**—Cover the diaphragm or mirror so that no transmitted light (§ 7), can reach the preparation, using the same preparation as in (§ 38). The oil and air will appear like globes of silver on a dark ground. The part that was darkest in each

* It should be remembered that the image in the compound microscope is inverted (Fig. 2), hence the bright spot really moves toward the mirror for air, and away from it for oil.

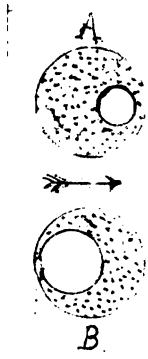

FIG. 5.—Diagram showing the position of the light spot in an *air bubble* (A), and an *oil globule* (B), when examined with oblique light. The arrow indicates the direction of the light.

will be lightest, and the bright central spot will be somewhat dark.*

(§ 40) **Distinctness of Outline.**—This depends on the difference between the refractive power of a body and that of the medium which surrounds it. The oil and air were very distinct in outline as each differed greatly in refractive power from the medium which surrounded them, the oil being more refractive than the mucilage and the air less.

Place a fragment of a cover-glass on a clean slide, and cover it (Hist. Notes § 6). The outline will be very distinct with the unaided eye. Use it as object and employ 3-4 objective and C ocular. Light with central light. The fragment will be outlined by a dark band. Put a drop of water at the edge of the cover-glass. It will run in and immerse the fragment. The outline will still be distinct, but the dark band will be somewhat narrower. Remove the cover glass, wipe it dry, and wipe the fragment and slide dry also. Put a drop of glycerin on the middle of the slide and mount the fragment of cover-glass in that. The dark contour will be much narrower than before.

Draw a solid glass rod out to a fine thread. Mount one piece in air, and the other in glycerin. Put a cover-glass on each. Employ the same optical arrangement as before. Examine the one in air first. There will be seen a narrow, bright band, with a wide dark band on each side.

The one in glycerin will show a much wider bright central band, with the dark borders correspondingly narrow.

If the glass rod or any other object were mounted in a medium of the same color and refractive power, it could not be distinguished from the medium.

(§ 41) **"Highly Refractive."**—This expression is often used in describing microscopic objects, (medullated nerve fibres for example), and means that the object will appear to be surrounded by a wide, dark margin when it is viewed by transmitted light. And from the above (§ 40), it would be known that the refractive power of the object, and the medium in which it was mounted must differ considerably.

(§ 42) **Doubly Contoured.**—This means that the object is bounded by two, usually parallel dark lines with a lighter band between them. In other words the object is bordered by (1) a dark line, (2) a light band, and (3) a second dark line.

This may be demonstrated by coating a fine glass rod (§ 40), with one or more coats of collodion or celloidin and allowing it to dry, and

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* It is possible to distinguish oil and air optically only when they are in small spherical masses. Oil and fatty substances may be distinguished chemically, no matter what their form, by the use of osmic acid, which blackens them (Hist. Notes, § 77).

then mounting in glycerin as above (§ 40). Employ a 1-5 or higher objective, light with transmitted light, and it will be seen that where the glycerin touches the collodion coating there is a dark line—next this is a light band, and finally there is a second dark line where the collodion is in contact with the glass rod.

(§ 42a) **Optical Section.**—The appearance obtained in examining transparent or nearly transparent objects with a microscope when some plane below the upper surface of the object is in focus. The upper part of the object which is out of focus obscures the image but slightly. By changing the position of the objective or object, a different plane will be in focus and a different optical section obtained. The most satisfactory optical sections are obtained with high objectives having wide angles. (See § 69).

Nearly all the transparent objects studied may be viewed in optical section. A striking example will be found in studying mammalian red blood-corpuscles on edge (Hist. Notes, § 13, 14).

(§ 43) **Currents in liquids.**—Employ the 3-4 objective, and as object put a few particles of carmine on the middle of a slide, and add a drop of water. Grind the carmine well with a scalpel blade, and then cover it. If the microscope is inclined, a current will be produced in the water, and the particles of carmine will be carried along by it. Note that the particles seem to flow up instead of down. This is due to the inversion of the image (Fig. 2).

(§ 44) **Pedesis or Brownian movement.**—Employ the same object as above, but a 1-5 or higher objective in place of the 3-4. Make the body of the microscope vertical, so that there may be no currents produced. Light the field well, using a small diaphragm. Focus, and there will be seen in the field large motionless masses, and between them small masses in constant motion. This is an indefinite dancing or oscillating motion.

This indefinite but continuous motion of small particles in a liquid is called *Pedesis or Brownian movement.* It is exhibited by all solid matter, if finely enough divided and in a suitable liquid. [(C., 182, 183), Journal of Science, 1878, p. 167)].

Compare the pedetic motion with that of a current by inclining the body of the microscope. The small particles will continue their independent leaping movements while they are carried along by the current.

(§ 45) **Demonstration of Pedesis with the Polarizing Microscope.**—The following demonstration shows conclusively that the pedetic motion is real and not imaginary. [R., 173].

Open the abdomen of a dead frog (an alcoholic specimen will do); cut out one of the spinal nerves close to its origin, being careful to

preserve with the nerve the small white sac which is upon it at its point of emergence from the vertebral foramen. Press this white sac on a slide in a drop of water. The water will become milky from the great number of minute crystals of carbonate of lime from the sac. Cover the preparation, and examine it with a 1–5 objective, C ocular. The smaller crystals will be seen to exhibit the pedetic movement.

Put the polariscope on the microscope and shade the stage by wrapping a black cloth around it; light the field as brilliantly as possible; shade the eye so that no light can enter it except through the microscope; focus sharply, then cross the polarizer and analyzer, that is, turn them in such a position that the field will be dark.

Part of the large crystals will shine continuously but the small moving ones will flash and disappear like meteors in a dark sky.

[General references to the interpretation of appearances (**B1**, 79, 244), (**C.** 178), (**Frey** 95), (**R**, 12), (**J. r. m. s.** 1882, p. 147), (**Popular Science Monthly**, Vol. VII, p. 177), (**LeConte**)].

MAGNIFICATION AND OCULAR MICROMETER RATIO.

(§ 46) The magnification, amplification or magnifying power of a microscope or any of its parts is the number obtained by dividing any linear dimension of the image by the corresponding linear dimension of the object magnified. For example, if the image of some object is 40 mm. long, and the actual length of the object magnified is 2 mm. the magnification is $40 \div 2 = 20$.

(§ 47) Magnification is expressed in diameters or times linear—that is but one dimension is considered.

(§ 48) **Distance at which the image is measured.**—While the image may be measured and the magnification determined at various distances, it is evident (Figs. 1, 2), that the size of the image, and hence the magnification, depends directly upon the distance at which it is measured from the apex of the triangle enclosing it, hence, in order that results may be uniform and comparable, it is necessary to agree upon some arbitrary distance at which the image shall be measured. The distance chosen is 25 cm. (10 inches approximately).

(§ 49) **Magnification of a simple Microscope.**—This is the ratio between the object magnified (Fig. 1, A B.), and the virtual image (Fig. 1, A′ B′). To obtain the size of the image (Fig. 1, A′ B′), place the tripod magnifier near the edge of a support of such a height that the distance from the upper surface of the magnifier to the table is 25 cm.

As object, place a scale of some kind ruled in millimeters on the support under the magnifier. Put some white paper on the table at the base of the support, and on the side facing the light.

Close one eye, and hold the head so that the other will be near the upper surface of the lens. Focus if necessary to make the image clear (§ 17). Open the closed eye, and the image of the rule will appear as if on the paper at the base of the support. Hold the head very still, and, with dividers, get the distance between any two lines of the image. This is the so-called method of binocular or double vision in which the microscopic image is seen with one eye and the dividers with the other, the two images appearing to be fused.

(§ 50) *The spread of the dividers* should be measured on a rule divided to 1-2 or 1-5 mm. This will give the size of the image as seen at 25 cm.

As 1-5 mm. cannot be seen plainly by the unaided eye, place one arm of the dividers at a centimeter line and then with the tripod magnifier count the number of spaces on the rule included between the points of the dividers. The magnifier simply makes it easy to count the spaces on the rule included between the points of the dividers—it does not, of course, increase their number or change their value.

Divide the size of the image by the size of the object magnified, the quotient will represent the power. Thus, suppose the distance between two lines of the image is 10.5 mm., and the actual distance on the rule is 2 mm., then the linear enlargemeut or the power is $10.5 \div 2 = 5.25$. That is, the image is 5.25 times as long as the object, and the instrument would be said to magnify 5.25 diameters, or 5.25 linear.

The power of any simple microscope is determined experimentally precisely as directed for the tripod. [C., 206, (Car. 68), (N. & S. 252)].

(§ 51) **Magnification of a compound microscope.**—This is the ratio between the final or virtual image (Fig. 2), obtained by the microscope and the object magnified.

(§ 52) **Stage micrometer,** *object or objective micrometer.*—This is a scale or rule upon glass. For all but the lowest objectives it is necessary to have a scale upon a transparent substance and the lines must be very fine. The most convenient form of a stage micrometer has part of the divisions in 1-10ths and part in 1-100ths mm.*

(§ 53) **Determination of the magnification of a compound microscope.**—**A.** *As with the simple microscope.* This is a very unsatisfactory and fatiguing method, and should be used only when one is unprovided with some form of camera lucida.

B. *With Wollaston's camera lucida* (Fig. 6, § 66).

Employ the 3-4 objective, A ocular and stage micrometer as object.

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* It is often difficult to find the band of lines on the micrometer, hence it is desirable to make a small ring of Brunswick black or other cement on the micrometer that shall just enclose the width of the band of rulings. This is not difficult, as the entire band may be seen with the unaided eye.

Focus sharply, and make the body of the microscope horizontal, by bending the flexible pillar, being careful not to bring any strain upon the fine adjustment (§ 24).

Put the camera lucida in position, and turn the ocular around if necessary so that the broad flat surface may face directly upward as shown in Fig. 6. Elevate the microscope by putting a block under the base, so that the perpendicular distance from the upper surface of the camera lucida to the table is 25 cm. Place some white paper on the work table beneath the camera lucida.

Close one eye, and hold the head so that the other may be very close to the camera lucida. Look directly down. The image will appear to be on the table. It may be necessary to readjust the focus after the camera lucida is in position. Measure the image with dividers and obtain the power exactly as above (§§49-50).

Put the C ocular in place of the A. Focus, and then put the camera lucida in position. Measure the size of the image with dividers and a rule as before. The power will be considerably greater than when the A ocular was used. This is because the virtual image (Fig. 2), seen with the high ocular is larger than the one seen with the low one. The real image (Fig. 2), remains nearly the same.

Put the 1-4 or higher objective in place of the 3-4; use the A ocular, focus, and then put the camera lucida in position. Get the size of the image. It will be much greater than when the 3-4 was employed. This is because the higher objective forms a larger real image (Fig. 2). As the A ocular magnifies the same as with the 3-4, the increase in size of the image must be wholly due to the greater size of the real image formed by the higher objective.

Lengthen the body of the microscope 5-6 cm. by pulling out the draw-tube. Put the 3-4 objective in position, remove the camera lucida, and focus, then replace the camera, and obtain the magnification. It will be greater than with the 3-4 objective and shorter body. This is because the real image (Fig. 2) is formed farther from the objective when the body is lengthened, and being formed farther from the objective it must necessarily be larger.

(54) **Varying the Magnification of a Compound Microscope.**—It will be seen from the above experiments (§ 53), that independently of the distance at which the microscopic image is measured (§ 48), there are three ways of varying the power of a compound microscope. These are named below in the order of desirability.

(1) *By using a higher or lower objective.*

(2) *By using a higher or lower ocular.*

(3) *By lengthening or shortening the body of the microscope.*

[References to the magnification of the compound microscope: (**B1**, 41, 355), (**C.** 161, 206), (**Car.** 68), (Fol. 58), (**N. & S.** 176), (**R.** 29), (**Robin** 126), (**Sat.** 7), (**A. s. m.**, Vol. VI., 183)].

(§ 55) **Ocular micrometer ratio.**—This is the quotient obtained by dividing the size of the real image (Fig. 2), as measured by an ocular micrometer, by the size of the object magnified.

(§ 56) **Ocular micrometer,** *Eye-piece micrometer.*—This, like the stage micrometer, is a piece of glass with parallel and equidistant lines, which are usually 1-5 to 1-20 mm. apart; they are coarser than the lines of the stage micrometer, so that they may be clearly seen by the low magnification of the ocular.

The ocular micrometer is placed in the ocular at the level of the real image (Fig. 2), and the real image appears to be immediately upon or under the ocular micrometer, and hence it can be easily measured.

(§ 57) **Obtaining the ocular micrometer ratio.**—As an example, employ the C ocular and 3-4 objective. Place the stage micrometer under the microscope for an object, and put the ocular micrometer in position.

Light the field well, and look into the microscope. The lines on the ocular micrometer should be very sharply defined. If they are not, raise or lower the eye-lens to make them so; that is, focus as with the simple magnifier.

When the lines of the ocular micrometer are distinct, focus the microscope (§ 19) for the stage micrometer. The image of the stage micrometer will appear to be directly under or upon the ocular micrometer.

Make the lines of the two micrometers parallel by rotating the ocular, or changing the position of the stage micrometer, or both if necessary, and then make any two lines of the stage micrometer coincide with any two on the ocular micrometer. To do this it may be necessary to pull out the draw-tube a greater or less distance. See how many spaces are included on each of the micrometers.

Divide the number of spaces on the ocular micrometer required to measure the image of the included spaces on the stage micrometer, by the value of the included spaces on the stage micrometer, and the quotient will be the ocular micrometer ratio. For example, suppose the stage micrometer is divided into one-tenths and one-hundredths mm., and that the image of two of the one-tenths is measured by five divisions on the ocular micrometer, then $5 \div \frac{2}{10} = 25$. That is, the ratio is 25. Or the value of each division on the ocular micrometer represents 1-25 mm. on the stage micrometer, for if 5 divisions on the ocular micrometer equal 2-10 mm. on the stage micrometer, 1 division on the ocular micrometer must equal 1-5 of 2-10 mm. or 1-25 mm. on the stage micrometer.*

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*For all the purposes of micrometry (§ 60-64), it is necessary to know only the *relative* value of the divisions of the ocular micrometer,

(§ 58) **Varying the ocular micrometer ratio.**—Any change in the objective, the ocular or the distance at which the real image is formed, that is to say, any change in the size of the real image, produces a corresponding change in the ocular micrometer ratio.

(§ 59) **Table of magnifications and ocular micrometer ratios.**—The following table should be filled out by each student, and in using it for future reference it is necessary to keep clearly in mind the conditions that produce variations in the magnification and the ocular micrometer ratio (§ 48, 54, 58):

	OCULAR A.		OCULAR C.		
OBJECTIVE.	TUBE IN.	TUBE OUT ——MM.	TUBE IN.	TUBE OUT ——MM.	OC. MICR. RATIO. TUBE——
3-4	×	×	×	×	
1-5	×	×	×	×	
1-8	×	×	×	×	
SIMPLE MICROSCOPE.		×			

MICROMETRY.

(§ 60) **Micrometry** is the determination of the size of objects by the aid of a microscope.

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but if it is desired to know the size of the real image in absolute measure, it is necessary to know the absolute value of the divisions on the ocular micrometer. Thus in the example given above, if the divisions on the ocular micrometer are 1-10 mm. then the real image is 5-10 mm. long, and the object being 2-10, the real image is 5-10 ÷ 2-10 = 2½ times the size of the object. This also represents the magnification of the objective as modified by the field lens in negative oculars, i. e., oculars in which the real image is formed between the eye lens and field lens. (A positive ocular is one in which the real image is formed below the entire ocular—the entire ocular acting like a simple microscope). If the ocular were positive or the field lens removed, it would represent the magnification of the objective. In obtaining the actual size of an object, the size of the real image in millimeters may be divided by the magnification of the objective used (2½ in the example), or the number of divisions (regardless of their absolute value) on the ocular micrometer measuring the real image may be divided by the ocular micrometer ratio (25 in the example). The result will be the same in the two cases, and will be in millimeters. Thus, 5-10 mm. ÷ 2½ = 1-5 mm; 5 (divisions) ÷ 25 = 1-5 (i. e., millimeter) as the stage micrometer used in obtaining the ratio was in millimeters.

(§ 61) **Micrometry with a simple microscope.**—(**A**) Place the object to be measured under the simple microscope, and then with fine pointed dividers obtain the dimensions desired. Get the spread of the dividers on a fine rule, as described in (§ 50), and the number of divisions on the rule will represent the size of the object in millimeters or fractions of a millimeter.

(**B**) Place the object under the simple microscope, and obtain with the dividers the size of the image exactly as in obtaining the magnification of the simple microscope (§ 49). Divide the size of the image by the magnification, and the result will represent the actual size of the object. For example, take a mounted fly's wing as object. Suppose that the image of the width of the wing measures 4.2 mm., and that the magnification of the simple microscope is 7 diameters, then the image must be 7 times as wide as the object; therefore the object is 4.2 mm. ÷ 7 = .6 mm. in width.

(§ 62) **Unit of measure in micrometry.**—As most of the objects measured with the microscope are smaller than any of the named divisions of the *meter*, and the common or decimal fractions necessary to express the size would often be inconveniently cumbersome, *Harting* (1859), proposed the 1-1000 of a millimeter as the unit of measure in micrometry. In 1869 the name micron (pronounced mic'-ron), was proposed for this unit by *Listing*. This name has been almost universally adopted, as has also its abbreviation, the Greek μ. [(**Beh.** 130), (**Car.** 70), (**Fol.** 67)].

(§ 63) **Micrometry with the compound microscope.**—(**A**) *By dividing the size of the image by the magnification of the microscope.*—For example, employ the 1-5 objective and C ocular, and a Necturus' red blood-corpuscle as object. Obtain the size of the image with the camera lucida and dividers exactly as in obtaining the magnification of the microscope (§ 53). Divide the size of the image by the magnification, and the result will be the actual size of the blood-corpuscle. Thus, suppose the image of the long axis of the corpuscle is 18 mm. and the magnification of the microscope 400 diameters (§ 47), then the actual length of this long axis of the corpuscle is 18 mm. ÷ 400 = .045 mm. or 45 μ (§ 62).

(**B**) *By dividing the size of the real image by the ocular micrometer ratio.*—Use the same objective, ocular and object as before. Put the ocular micrometer in position, and count the divisions on the ocular micrometer required to include or measure the long axis of the blood-corpuscle.* Divide the number of divisions so

* It is somewhat difficult to count accurately the spaces on the ocular micrometer. To overcome this difficulty, a camera lucida may be used and the lines of the ocular micrometer and the outline of the object traced on paper. It is then easy to count the lines on the paper.

obtained by the ocular micrometer ratio, and the result will be the actual size in millimeters. Thus, suppose it requires 9 divisions on the ocular micrometer to include the blood-corpuscle, and the ocular micrometer ratio is 200, the actual size of the corpuscle is $9 \div 200 = .045$ mm. (§ 57), or 45 μ, as before.

(C) *By the use of a stage micrometer and a camera lucida.*—Employ the same object, objective and ocular as before. Put the camera lucida in position, and with a lead pencil make dots on the paper at the limits of the image of the blood-corpuscle.

Remove the object, place the stage micrometer under the microscope, focus well, and draw the lines of the stage micrometer so as to include the dots representing the limits of the part of the image to be measured. As the value of the spaces on the stage micrometer is known, the size of the object is determined by the number of spaces of the micrometer required to include it.

This simply enables one to put the image of a fine rule on the image of a microscopic object. It is an excellent method, and practically the same as measuring the spread of the dividers with a simple microscope (§ 50).

If the work is properly done the result will be the same by the different methods.

(§ 64) **General remarks on micrometry.**—(A) In using adjustable objectives (§ 70), the magnification of the objective varies with the position of the adjusting collar, being greater when the adjustment is closed as for thick cover-glasses than when open, as for thin ones. This variation in the magnification of the objective produces a corresponding change in the magnification of the entire microscope and the ocular micrometer ratio,—therefore it is necessary to determine the magnification and ocular micrometer ratio for each position of the adjusting collar.

(B) *The general precaution is to have all the conditions, when the unknown object is measured, precisely as when the power of the microscope or the ocular micrometer ratio was determined.*

(C) While the principles of micrometry are simple, it is very difficult to get the exact size of microscopic objects. This is due to the lack of perfection and uniformity of micrometers, and the difficulty in determining the exact limits of the object to be measured. Hence, all microscopic measurements are only approximately correct, the error lessening with the increasing perfection of the apparatus and the skill of the observer. [(**B1**, 43), (**Beh.** 120), (**C.** 109), (**R.** 32), (**N. & S.** 283), (**A. q. m. j.** Vol. I, pp. 97, 208). (See also references in Hist. Notes (§ 70c)].

DRAWING MICROSCOPIC OBJECTS.

(§ 65) Microscopic objects may be drawn free-hand directly from

the microscope, but in this way a picture giving only the general appearance and relations of parts is obtained. For pictures which shall have all the parts of the object in true proportions and relations, it is necessary to obtain an exact outline of the image of the object, and to locate in this outline all the principal details of structure. It is then possible to complete the picture free-hand from the appearance of the object under microscope. The appliance used in obtaining outlines, etc., of the microscopic image is known as a *camera lucida.*

(§ 66) **Camera lucida.**—This is an optical apparatus for enabling one to see, as if *in one field of vision and with the same eye*, objects in greatly different situations. As applied to the microscope, it causes the magnified virtual image of the object under the microscope to appear as if projected upon the table or drawing board, where it is visible with the drawing paper, pencils, dividers. etc., by the same eye, and in the same field of vision. The microscopic image appears like a picture on the drawing paper. This is accomplished in two distinct ways :

(**A**) By a camera lucida reflecting the rays from the microscope so that their direction when they reach the eye coincides with that of the rays from the drawing paper, pencils, etc. (Fig. 6). In some of the camera lucidas of this group (Wollaston's (Fig. 6), and Schrœder's), the rays are reflected twice and the image appears as when looking directly into the microscope. In others, the rays are reflected but once and the image has the inversion produced by a plane mirror. For drawing purposes this inversion is a great objection, as it is necessary to similarly invert all the details added free-hand.

(**B**) By a camera lucida reflecting the rays of light from the drawing paper, etc., so that their direction when they reach the eye coincides with the direction of the rays from the microscope (Fig. 7-8). In all of the camera lucidas of this group, the rays from the paper are twice reflected and no inversion appears.

The better forms of camera lucidas (Wollaston's, Grunow's, Abbe's, etc.), may be used for drawing both with low and with high powers. Some require the microscope to be inclined (Fig. 6), while others are designed to be used on the microscope in a vertical position (Fig. 7-8). As in biological work it is often necessary to have the microscope vertical, this form is to be preferred. [(**Bi**, 31, 355), (**Beh.** 110), (**C.** 112), (**Car.** 73), (**Fol.** 67), (**J. r. m. s.** nearly every volume). For drawing at a magnification of from 5 to 100 diameters for large objects see (**W.** 132), (**J. r. m. s.** 1881, p. 819; 1882, p. 402; 1884, p. 115), (**Amer. Naturalist**, 1886, p. 1071)].

(§ 67) **Avoidance of distortion.**—In order that the picture drawn by the aid of a camera lucida may not be distorted, it is necessary that the axial ray from the image on the drawing surface shall be at right angles to the drawing surface.

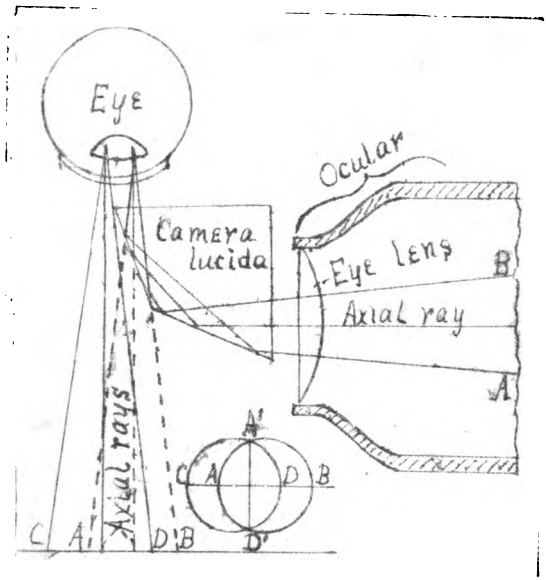

FIG. 6.—Diagram of a camera lucida (Wollaston's), which changes the direction of the rays from the microscope, the rays from the drawing surface remaining unchanged.

A–B.—Lateral or converging rays from the microscope, and their projection on the drawing surface at right angles to their original direction.

Axial ray.—The central ray of the field of vision. It is in the principal optical axis of the microscope. Its projection on the drawing surface is shown as a dotted line. An axial ray from the center of the field of vision on the drawing surface is also shown.

C–D.—Lateral or converging rays from the field of vision on the drawing surface.

C. L.—Camera lucida. This is a prism with a quadrangular base.

Eye.—Eye of the observer. Two independent cones of rays are received by the same eye. Using this camera lucida is like looking through two holes in a screen about one or two millimeters apart. The superimposed parts of the images may be seen at the same time. In the diagram the images are superimposed between A and D. See also superimposed circles at the right.

Eye lens of the ocular.

Ocular.—Part of the ocular or eye-piece of the microscope.

In Fig. 6 and 7 this is seen to be the case where the drawing surface is horizontal, but in some camera lucidas, especially those which reflect the image of the drawing paper and pencil (§ 66 B), the reflecting mirror or prism is so close to the ocular that the angle of incidence of the axial ray must be less than 45 degrees, in order that the image shall be free from the foot of the microscope (Fig. 8). In this case, the axial ray is not vertical, and if the drawing were made on a horizontal surface, the part of the picture farthest from the microscope would be considerably more magnified than the part next the microscope (Fig. 8). In order to avoid this distortion when the axial ray of the image is oblique, it is necessary to incline the drawing surface so that it shall form a right angle with the axial ray as in Fig. 8, A–B.

(§ 68) **Determination of the proper inclination of the drawing surface.**—In order to do this it is necessary first to locate the center of the field, and hence the position of the axial ray of the image. This may be done most satisfactorily by using a micrometer ruled in squares (net-micrometer) as object, and arranging it so that the crossing point of two lines shall be exactly in the middle of the field. If one does not possess a net-micrometer, some other small object with sharp outlines may be used, and put as nearly as possible in the center of the field. After a suitable object is in the center of the field, put the camera lucida in position, and incline the mirror or prism so that the image shall be in a convenient position for drawing, then take some rectangular block, with sides about 5 cm. long, and place it vertically on the drawing surface and with one corner exactly in the center of the field—that is at the point where the image of the object which was placed in the center is formed—then slowly incline the rectangular block until the image of the central object is exactly opposite the upper corner as well as the lower one. This will occur only when the straight edge of the block connecting the two corners is parallel with the axial ray. The angle of inclination of the block (Fig. 8 e C f), indicates the inclination necessary for the drawing surface (Fig. 8 B C B′).*† [(J. r. m. s. 1883, pp. 560–567), (Fol. p. 70)].

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* When the block is placed on the drawing surface, and one of its corners made to coincide in position with the center of the field, the block will appear as if inclined in the opposite direction from the axial ray, if the axial ray and the drawing surface are not at right angles. Also when the drawing board is properly inclined, the block will appear at an angle if it is moved away from the center of the field. This is readily intelligible from the figure, where it is seen that only the central or axial ray of the image is perpendicular to the drawing surface, all the others are inclined toward the axial ray.

† If the objective used does not have a flat field, the image at the edges will be more magnified than at the center, independently of the drawing surface;—hence it is better to draw all objects as near the center of the field as possible. (**N. & S.**, p. 59.)

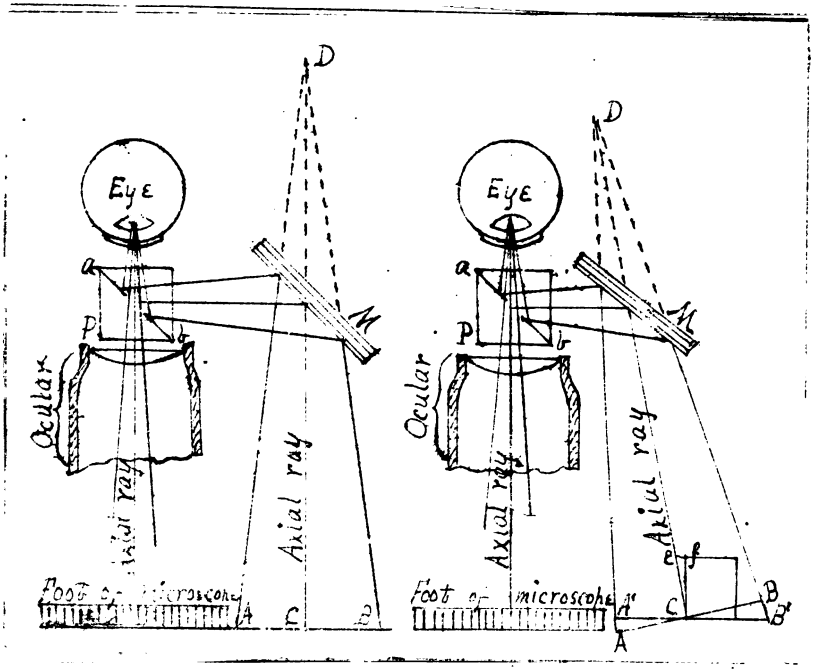

FIG. 7–8.—Digrams of two camera lucidas which change the direction of the rays from the drawing surface, the direction of the rays from the microscope remaining unchanged.

A C B.—Drawing surface at right angles to the axial ray in Fig. 7–8.

A′ C B′.—Horizontal surface in Fig. 8. As the distances D A′ and A′ C are considerably less than the distances D B′ and C B′, the part of the figure near B′ would be considerably more magnified than the part near A′.

a b.—Silvered surface of the divided cubical prism for reflecting upward the rays from the mirror or prism M. The silvering is removed from the central part so that the rays from the microscope pass directly through to the eye.

Axial ray.—The ray from the center of the field of vision.

D.—Apex of the triangle including the field of view on the drawing surface. In obtaining the magnification of a microscope with such a camera lucida as this, the 25 cm. should be measured from the point D along the line of the axial ray. Or, as this point would be difficult of determination, the distance may be approximately obtained by measuring from the edge of the cubical prism a–P. to the center of the reflecting mirror (M) and thence downward, following the line of the axial ray.

Eye.—Eye of the observer.

Foot of microscope.—See Fig. 3.

M.—Mirror or prism reflecting the rays from the paper to the prism over the ocular.

Ocular.—Part of the ocular or eye-piece of the microscope. (See Fig. 2).

P.—Cubical prism over the eye-lens of the ocular.

(§ 69) **Drawing with a camera lucida.**—In order that both the microscopic image and the drawing paper, pencil, etc., may be clearly seen, it is necessary to have the lighting of the field of the microscope and the drawing paper properly balanced. Proper lighting with each form of camera lucida must be learned by experience. Depending upon the object to be drawn, the field of the microscope may need to be strongly or weakly lighted or the drawing paper, pencils, etc., may be strongly or weakly lighted. If the drawing is upon light paper the pencil point should be made as black as possible by coating it with India ink. Sometimes it is easier to draw on a black surface, using a white pencil or style. The carbon paper used in manifolding letters, etc., may be used, or ordinary black paper may be lightly rubbed on one side with a moderately soft lead pencil. Place the black paper over white paper and trace the outlines with a pointed style of ivory or bone. A corresponding dark line will appear on the white paper beneath. (J. r. m. s. 1883, p. 423).

(A) It is desirable to have the drawing paper fastened with thumb tacks, or in some other way. (B) The lines made while using the camera lucida should be very light, as they are liable to be irregular. (C) It is desirable to draw the image of a few of the spaces of a stage micrometer near each drawing. The actual value of the spaces should be given, and all the conditions should be exactly as when making the drawing of the object. (D) If a drawing of a given size is desired and it cannot be obtained by any combination of oculars, objectives and lengths of the body of the microscope, the distance between the camera lucida and the table may be increased or diminished until the image is of the desired size. The image of a few spaces of the micrometer, as in (C), will give the scale of enlargement, or the power may be determined for the special case. (E) It is sometimes desirable to draw the outline of an object with a moderate power and add the details with a higher power. If this is done it should always be clearly stated. It is advisable to do this only with objects in which the same structure is many times duplicated, as a nerve or a muscle. In such an object all the different structures could be shown, and by omitting some of the fibers the others could be made plainer without an undesirable enlargement of the entire figure. [(Bı, 31, 355), (Beh. 110), (C. 112), (Fol. 70), (Frey 38), (J. r. m. s. 1883, pp. 283, 560; 1886, 516), (Z. w. m. 1884, p. 1–21)].

ADJUSTABLE, IMMERSION, AND APOCHROMATIC OBJECTIVES.

(§ 70) **Adjustable and non-adjustable objectives.**—An adjustable objective is one in which the distance between the systems of lenses (usually the front and the back systems) may be changed by the observer at pleasure. The object of this adjustment is to correct or compensate for the displacement of the rays of light produced by the mounting medium and the cover-glass after the rays have left the object. It is also to compensate for variations in "tube length." See § 73. As the displacement of the rays by the cover-glass is the most constant and important, these objectives are usually designated as having cover-glass adjustment or correction.

Non-adjustable objectives are those in which the lens systems are fixed and no compensation can be made for the various accidental displacement of the rays from the object, by changing the relative position of the lens systems; but as cover-glasses are used for almost all objects, opticians arrange the systems so that they will give the best image of mounted objects with cover-glasses of medium thickness. The exact thickness of cover-glass chosen by several opticians is here given, by their courtesy:

For covers 0.16 mm., Bausch & Lomb Opt. Co.
" " 0.25 " Green, successor to Tolles.
" " 0.25 " Grunow.
" " 0.15 " Gundlach Opt. Co.
" " 0.25 " H. R. Spencer & Co.
" " 0.15–0.20 mm., Zeiss.
" " 0.16 mm., Zeiss' homogenous apochromatic objectives (§ 76).

For beginners, and those unskilled in using adjustable objectives, the non-adjustable ones give the most satisfactory results, but for the highest kind of microscopical work it is desirable that the objectives be adjustable so that compensation may be made for the imperfections in the image, due to unavoidable variations in mounting media, cover.glasses and "tube-length."

When non-abjustable objectives are used, one should adhere as closely as practicable to the thickness of cover-glass and the "tube-length" for which they were corrected.

(§ 71) **Adjustment for objectives.**—(A) *Non-adjustable objectives.*—Any slight variation in the thickness of the cover, the character of

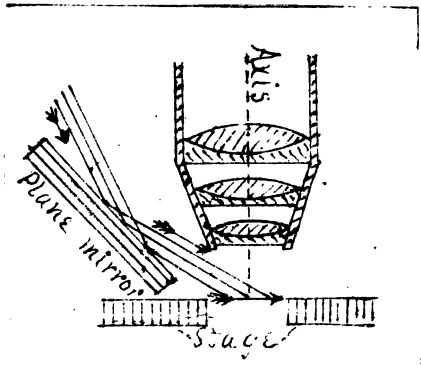

FIG. 9.—Diagram of reflected light and of a dry, non-adjustable objective in section.

Axis.—The principal optical axis of the microscope. It is along this axis that the central ray from the field of vision extends.

Plane mirror.—The mirror is represented as above the stage, and light is reflected upon the object—the horizontal arrow. The other arrows simply indicate the direction of the rays of light.

Stage.—The stage of the microscope in section (Fig. 3). The object is over the aperature in the stage.

the mounting medium or the standard "tube-length," produce such slight disturbance that they may be neglected, especially with low objectives; but if the variation is considerable, compensation may be made as follows: For cover-glasses thinner than the standard, *increase* the "tube-length," for thicker ones *diminish* it. See also "Tube-length" (§ 73).

(B) *Adjustable objectives.*—The proper adjustment of objectives, that is, the adjustment which gives the truest image, requires both insight and experience; for the structure of an object does not appear the same with different adjustments of the objective. And as the opinion of different observers on the structure of objects varies, they adjust the objectives differently, and try to obtain the adjustment which will show a structure in accordance with their opinion. Eyes also differ, and two observers might find it necessary to adjust the same objective differently to produce an identical appearance for each of them.

In learning to adjust objectives, it is best for the student to choose some object whose structure is well agreed upon, and then to practice lighting it, shading the stage and adjusting the objective, until the proper appearance is obtained. The adjustment is made by turning a ring or collar which acts on a screw and increases or diminishes the distance between the systems of lenses, usually the front and the back systems (Fig. 10). In adjustable objectives the back system should be movable, the front one remaining fixed so that there will be no danger of bringing the objective down upon the object. If the front system is movable, the body of the microscope should be raised slightly every time the adjustment is altered.

General directions.—(A) The thinner the cover-glass the further must the systems of lenses be separated, i. e., the adjusting collar is turned nearer the zero or the mark "uncovered," and conversely. (B) The thicker the cover-glass, the closer together are the systems brought by turning the adjusting collar *from* the zero mark. This also increases the magnification of the objective (§ 46, 58, 64).

The following specific directions for making the cover-glass adjustment are given by Mr. Wenham [(C. 166), (Q. j. m. s. 1854, p. 137)]: "Select any dark speck or opaque portion of the object, and bring the outline into perfect focus; then lay the finger on the milled-head of the fine motion, and move it briskly backwards and forwards in both directions from the first position. Observe the expansion of the dark outline of the object, both when within and when without the focus. If the greater expansion, or coma, is when the object is *without* the focus, or farthest from the objective [i. e., in focusing up], the lenses must be placed farther asunder, or toward the mark uncovered [i. e., the adjusting collar is turned toward the zero mark as the

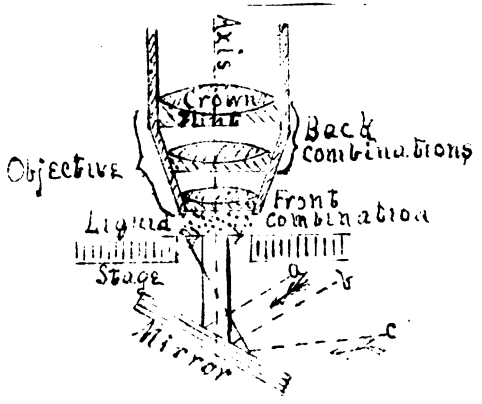

FIG. 10.—Diagram of an adjustable, immersion objective and of transmitted light.

Axis.—The principal optical axis of the microscope.

a b.—Central or axial light, so called because parallel to the principal optical axis of the microscope, after reflection from the mirror.

Back Combinations.—The two back systems or combinations of lenses in the objective.

Crown.—The double convex lens of crown glass forming part of each combination.

Flint.—The plano-concave lens of flint glass forming part of each combination.

c.—Oblique light, so called because after reflection from the mirror the ray forms an angle with the principal optical axis of the microscope.

Front combination.—The front system or combination of lenses in the objective.

Liquid.—This is between the front combination and the object or the cover-glass. It may be water, glycerin or oil, according to the construction of the objective.

Mirror.—The mirror is below the stage and reflects light up through the object.

Objective.—This comprises the combination of lenses in their mounting.

Stage.—The stage of the microscope in section (Fig. 3).

Object.—An arrow. It is over the aperature in the stage.

cover-glass is too thin for the present adjustment]. If the greater expansion is when the object is within the focus, or nearest the objective, [i. e., in focusing down], the lenses must be brought closer together or toward the mark covered [i. e., the adjusting collar should be turned away from the zero mark, the cover-glass being too thick for the present adjustment]." In most objectives the collar is graduated arbitrarily, the zero (0) mark representing the position for uncovered objects. Other objectives (Zeiss' and Leitz', also some by Powell & Leland) have the collar graduated to correspond to the various thickness of cover-glasses for which the objective may be adjusted. This seems to be an admirable plan; then if one knows the thickness of the cover-glass on the preparation (Hist. Notes, § 25, note), the adjusting collar may be set at a corresponding mark, and one will feel confident that the adjustment will be approximately correct. It is then only necessary for the observer to make the slight adjustment to compensate for the mounting medium or any variation from the standard length of the body of the microscope. In adjusting for variations of the length of the body from the standard it should be remembered that: (A) If the body of the microscope is longer than the standard for which the objective was corrected, the effect is approximately the same as thickening the cover-glass, and therefore the systems of the objective must be brought closer together, i. e., the adjusting collar must be turned *away from* the zero mark. (B) If the body is shorter than the standard for which the objective is corrected, the effect is approximately the same as diminishing the thickness of the cover-glass, and the systems must therefore be separated, that is, the adjusting collar turned *toward* the zero mark.

[References to adjustable objectives and to their manipulation: [(**Bi**, 10), (**Bau.** 66), (**Beh.** 32), (**C.** 165), (**Fol**, 34), (**Frey**, 16, 58), (**H.** 63), (**N. & S.** 57), (**R.** 22), (*Richardson*, p. 61), (*Encyc. Brit.* article microscope), (**A. s. m.** 1881, p. 61, 1884, pp. 216, 218, 273), (**Q. j. m. s.** 1854, p. 137, 1861, p. 87), (*Amer. Jour. Science*, June 1872), (**J. r. m. s.** 1884, p. 620), (*Amer. Jour. Med. Sci.* Jan. 1875, p. 158), (**Z. w. m.** 1884, p. 29, 1885, p. 73), (*Transactions of the Society of Arts*, 1837, p. 99)].

(§ 71) **Angular and numerical aperature of objectives.**—For a full discussion of this subject see: [(**C.** 850), (**H.** 69), (**A. s. m.** 1884, pp. 5–39), (**J. r. m. s.** 1881, pp. 303, 348, 365, 388; 1882, pp. 300, 460; 1883, p. 790; 1884, p. 20)].

(§ 73) **"Tube length."**—This means, generally speaking, the distance between the ocular and objective. The exact significance of "tube length," its length in millimeters, and the parts included in

it, vary with different opticians. This variation is shown in Fig. 11.*

Whatever the interpretation by different opticians of what should be included in "tube length," and the exact length in millimeters, its importance is very great; for each objective gives the most perfect image of which it is capable with the "tube length" for which it is corrected, and the more perfect the objective the greater the ill effects on the image of varying the "tube length" from this standard. The plan of designating exactly what is meant by "tube-length," and engraving on each objective the "tube-length" for which it is corrected, is to be commended, for it is manifestly difficult for each worker with the microscope to find out for himself for what "tube-length" each of his objectives was corrected.

(§ 74) **"Optical tube-length."**—This expression is used especially by Abbe to designate the distance measured along the axis of the microscope, between the points where paralled rays passing from the outside of the microscope would be brought to a focus by the objective and ocular respectively. [(J. r. m. s. 1883, pp. 790, 816; 1884, pp. 450, 811), (Z. w. m. 1886, p. 314), (M. B. 1884, pp. 25, 37)].

(§ 75) **Dry and immersion objectives.**—A dry objective is one in which the space between the front of the objective and the object or the cover-glass is filled with air (Fig. 9).

An immersion objective is one in which the space between the front of the objective and the object or cover-glass is filled by some liquid (the immersion liquid) (Fig. 10).

Homogeneous immersion objectives.—With these the immersion liquid (some kind of oil or oily substance, or glycerin with a salt in solution), closely resembles glass in its effect on the light, and hence the effect is practically as if the objective were in optical contact with the object. Adjustment for these objectives is necessary for the slight displacement of the rays by the mounting medium and for variations in the body of the microscope from the standard for which the objective was corrected. According to the opinion of many, the disadvantages of having these objectives abjustable outweigh the advantages.

Immersion objectives, in which the immersion fluid is water or glycerin, are usually adjustable. The advantages arising from the use of immersion objectives are very great, the image being far clearer than with dry objectives of the same grade.

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*I have to thank the opticians named for the information given in connection with Fig. 11. The meaning of "tube length" as used by Zeiss was taken from his catalogues and from Dippel. [(Z. w. m. 1886, p. 311) and (J. r. m. s. 1886, p. 849)].

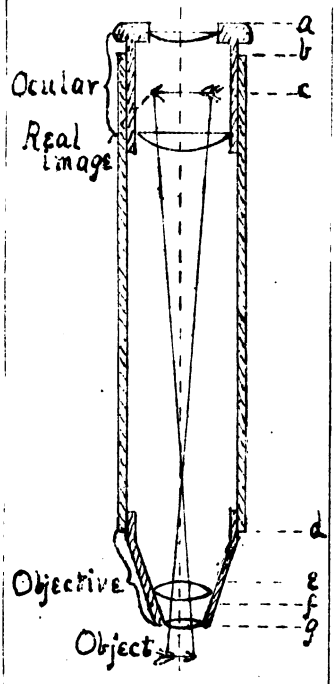

FIG. 11.—Diagram to illustrate what is included in "tube-length" by various opticians. See also § 73.*

a–g.—(254 mm.), Gundlach Optical Co. (Both ocular and objective are included in the distance known as "tube-length").

b–d.—(160 or 250 mm.), Carl Zeiss. "The 'tube-length' is measured from the upper surface of the setting of the objective to the upper margin of the body-tube, on which the eye-piece rests." (J. r. m. s. 1886, p. 850, Z. w. m. 1886, p. 311).

b–d.—(203 mm.), J. Grunow. (The distance between the lower end of the body where the objective is applied, and the top of the body, when the ocular extends in, as in the figure, or to the mounting of the eye-lens if the ocular is after the English pattern).

c–g.—(254 mm.), H. R. Spencer & Co. (The distance from the front of the objective to the field-bar of the eye-piece).

c–f.—(254 mm.), J. Green, successor to Tolles. (The distance from the front shoulder of a three system objective, or about midway between the systems of a two system objective, to the focus of the eye-lens of the ocular).

c–e.—(216 mm.), Bausch & Lomb Opt. Co. (The distance from the focal plane of the objective to the diaphragm in the ocular).

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*The information concerning "tube-length" as used by American opticians, was kindly furnished by them; and to make sure that there should be no misinterpretation, a diagram, like Fig. 11, was sent to each with the request that the parts included be indicated on the diagram.

Care of immersion objectives.—There should be used only enough of the immersion liquid to fill the space between the front of the objective and the cover-glass. An excellent way to apply it is to dip a clean glass rod in the immersion liquid and then touch the adherent liquid to the end of the objective. When through working, the immersion fluid should be carefully removed from front of the objective. The best means of doing this is to use a clean piece of the Japanese bibulous paper (§ 25). It is well also to wipe occasionally the front of the objective, especially a homogenous one, with the paper moistened with 95 per cent. alcohol (§ 25).

It sometimes happens, especially with homogeneous objectives, that when a strong light strikes the top of the stage and the lower end of the objective the image is not clear. Shading the objective will remedy this (§ 10).

[References to immersion objectives: (**B**1, 8, 435), (**Bau.** 66), (**Beh.** 30), (**C.** 18, 20), (**Car.** 45), (**Fol,** 36), (**Frey** 58–60), (**H.** 81, 86), (*Mayall*, p. 95), (**R.** 23), (*Science Lectures at South Kensington*, Vol. I, p. 210), (**A. s. m.** 1881, p. 61; 1884, p. 32), (**J. r. m. s.** nearly every volume), (**M. B.** 1885, p. 22). See also the references to adjustable and apochromatic objectives (§§ 70, 76)].

(§ 76) **Apochromatic objectives.**—This name has been given by Zeiss to objectives made of new kinds of glass. They are made adjustable and non-adjustable, dry, and for water and homogeneous immersion liquids. Their special features are:

(1) *Three* rays of different color are brought to one focus, leaving a small tertiary spectrum only, while with objectives as formerly made from crown and flint glass, only *two* different colors could be brought to the same focus.

(2) In these objectives the correction of the spherical aberration is obtained for *two* different rays of the spectrum, that is practically for all the colors at the same time and the objective shows the same degree of chromatic correction for the marginal as for the central part of the aperture. In the old objectives, correction of the spherical aberration was confined to rays of *one* color, the correction being made for the central part of the spectrum, the objective remaining *under*-corrected spherically for the red rays and *over*-corrected for the blue rays.

(3) The optical and chemical foci are identical, and the image formed by the chemical rays is much more perfect than with the old objectives, hence the new objectives are well adapted to photography.

Special oculars—*compensating oculars*—are used with the objectives "to compensate for certain aberrations outside the axis, which cannot be compensated for in the objective." The objectives admit

of the use of very high oculars, and seem to be in every way a great improvement over those made in the old way with crown and flint glass. According to Dippel (**Z. w. m.** 1886, p. 300), dry apochromatic objectives give as clear images as the same power water immersion objectives of the old form.

In using the apochromatic objectives it is especially necessary that they be used with the "tube-length" for which they were corrected (160 or 250 mm., Fig. 11, b–d), and also that the adjustment for cover-glass be very carefully made. These objectives, which are for homogeneous immersion, are non-adjustable, and are corrected for cover-glasses 0.16 mm. thick. Slight variations from this thickness are of no practical consequence, but considerable variations should be corrected (a) by increasing the "tube-length" by pulling out the draw-tube for thinner cover-glasses, and by decreasing the "tube-length" for thicker ones. [(**Zeiss' catalogue**), (**J. r. m. s.** 1886, p. 849)].

The setting of the front of the high objectives (those of 2 and 3 mm. focus, 1.40 numerical aperture) is very thin, and it is necessary to use them with great care. At present the new objectives and oculars are made by Zeiss, of Germany, and Powell, of England, those of Powell being of somewhat simpler construction than those of Zeiss (**J. r. m. s.** 1886, p. 1110).

[References to the new kinds of glass and to the apochromatic objectives: (**J. r. m. s.** 1886, pp. 316, 849, 1110), (**Z. w. m.** 1886, 303), (**M. B.** 1886, pp. 18, 35, 43), (**M.** 1886, p. 112), (**A. m. m. j.** 1886, pp. 76, 88, 214, 231; 1887, p. 7)].

(§ 77) **Illuminators.**—For many purposes, and especially for high objectives, the light from a mirror directly upon the object is insufficient, hence there have been prepared various forms of optical instruments, either to condense the light upon the object or to light the object with rays of great obliquity. The illuminator or condensor now most in favor is the one devised by Abbe, and known by his name. For some purposes, as the examination of stained microbes, no diaphragm need be used, but where fine details of histological structure are to be studied, it is often advantageous to use a small diaphragm. It is often desirable also to move the illuminator nearer or farther from the object. The beginner should try with each preparation the illuminator, near to and far from the object, and with diaphragms of various sizes. After experience is gained in this way, the proper illumination can be decided upon very quickly as with the mirror alone. [(**B1**, 352), (**Bau.** 79), (**Beh.** 82), (**C.** 120)].

APPENDIX.

(§ 78) **Imbedding in celloidin.**—In the Histological Notes (§ 58, p. 30), directions are given to imbed objects in glass test-tubes and then after the celloidin is hard it is to be shaken from the tube. This has been found impracticable, and the following method is recommended: After the tissue is properly infiltrated, first in thin then in thick celloidin, make an imbedding cylinder by winding writing paper around a cork which has been previously seaked in celloidin. Arrange the object as desired and fill the cylinder with thick celloidin so as to immerse the object for a considerable depth. After a film has formed over the surface of the celloidin, the imbedded object is put into a bottle of chloroform. When the celloidin is hardened throughout, the paper is removed and the object is ready for sectioning. If as soon as the imbedded object is placed in chloroform the bottle is put in a cold place while the celloidin is hardening, air bubbles are less liable to form.

(§ 79) **Cutting celloidin sections.**—If the imbedded object is of considerable size it is a great advantage to cut the sections under alcohol. To do this, the entire microtome may be placed in a large galvanized iron box and covered with 75 per cent. alcohol. The microtome works more satisfactorily in every way under alcohol than in the air.

(§ 80) **Fastening celloidin sections to the slide.**—It has been found by Mr. H. E. Summers, B.S., Fellow in Comparative Anatomy, that the elaborate method given in the Hist. Notes, p. 31, is unnecessary. The simple method devised by him this winter is here given by permission, previous to its publication in the *Microscope:* Dehydrate the sections for a minute or two in 95 per cent. alcohol, then arrange them on the slide, and drain off the alcohol by tipping the slide. Now pour over the sections *vapor* of sulphuric ether from a bottle partly filled with liquid ether. The celloidin will almost immediately soften and become perfectly transparent. As soon as this occurs the slide may be placed in 80 per cent. or even 95 per cent. alcohol, and the sections washed with water, stained, dehydrated and mounted without any danger of their becoming loosened from the slide.

(§ 81) **Clearing.**—For clearing celloidin sections before mounting them in balsam, the mixture of carbolic acid and turpentine—melted crystals of carbolic acid 20 cc., turpentine 30 cc., Hist. Notes, § 94, p. 44—has proved very satisfactory.

(§ 82) **Counting white blood-corpuscles.**—White blood-corpuscles are so few in comparison with the red ones in mammalian blood that

it is somewhat difficult to count them unless they are strongly differentiated from the red ones. The following methods have been found to be excellent: Toison's method (J. r. m. s. 1886, p. 698). The diluting liquid contains a staining agent that colors the white corpuscles purple but does not affect the red ones. It is prepared as follows: Distilled water, 160 cc.; glycerin, 30 cc.; sodium sulphate, 8 grams; sodium chloride, 1 gram; methyl violet, 5 B. 0.025 grams. The methyl violet is dissolved in the glycerin and half the water, the salts in the other half of the water; then the two solutions are mixed and filtered after the mixture is cold, The blood is mixed with this in the usual proportions if both the red and the white corpuscles are to be counted. If only the white ones, the proportion of blood may be increased five or ten-fold. The staining action takes place in 20 to 30 minutes.

(**B**) Thoma's method: (**Ar. p. A.** Bd. 87, (1882), p. 201). A one-third per cent. aqueous solution of acetic acid is used as the diluent. Blood is mixed with this in the proportion of one to ten. The acetic acid makes the red corpuscles invisible, while it increases the visibility of the white ones; hence by this method only the white ones can be counted.

(§ 83) **Stronger cleaning mixture for glass.**—For cleaning slides and cover-glasses that have been used for balsam preparations or sealed glycerin preparations, the cleaning mixture given in the Histological Notes, § 72, p. 42, acts too slowly. A cleaning mixture containing only about one-fourth as much water has been found to clean old slides in about ten days, and new cover-glasses in about half an hour, although a prolonged stay in the mixture does no harm. The stronger cleaning mixture is prepared as follows:

Water	2000 cc.
Potassium dichromate	800 grams.
Strong commercial sulphuric acid	800 cc.

The water and potassium dichromate are mixed and heated till the dichromate is dissolved, then the sulphuric acid is added.

(§ 84) **Cleaning large cover-glasses.**—It is difficult to clean cover-glasses for serial sections, these being 48x60 mm. and in some cases larger. The most satisfactory methods employed at present are to soak the covers for several days in the ordinary cleaning mixture for glass or a less time in the stronger mixture (§ 83), then after all the cleaning mixture is rinsed away, to put upon the covers alcohol of 75 to 95 per cent., and then in wiping the covers they are laid on blotting paper and the surfaces carefully wiped with a clean, soft handkerchief, or preferably, two flat blocks somewhat larger than the cover-glasses are covered with several thicknesses of soft, clean cloth, and the covers are rubbed, one at a time, between these blocks.

SUPPLEMENTAL LIST OF REFERENCE BOOKS AND PERIODICALS.

The books and periodicals in the following list were omitted from the list given in the Histological Notes by inadvertence, or they were not at that time in possession of the laboratory or the university library. References are made in the text to this supplemental list and to the principal list as described in the prefatory note of the Histological Notes, q. v.

BOOKS.

Bernstein, J.—The five senses of man. Pp. 304, illustrated. New York, 1876.

Bz.—Bizzozero, G. *et* Firket, Ch.—Manuel de microscopie clinique, microscopie légale, chemie clinique, technique bactérioscopique. Pp. 557, illustrated. Paris et Bruxelles, 1885. Methods and structure.

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MICROSCOPICAL NOTES.

SIMON H. GAGE.

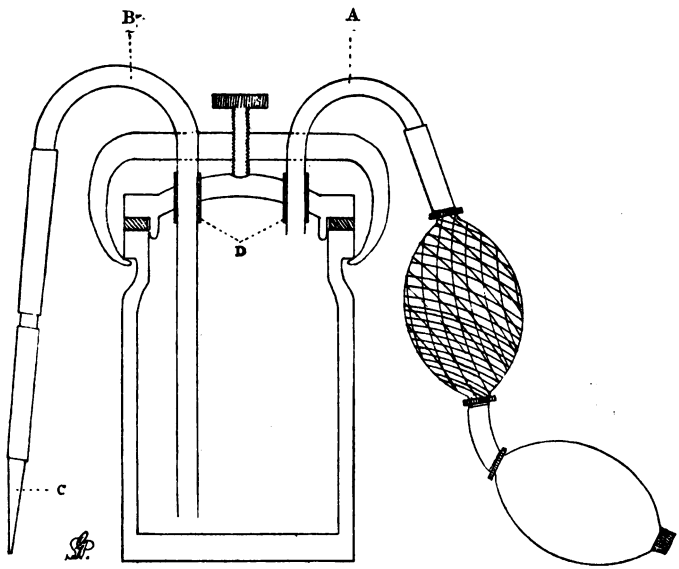

FIGURE 1. INJECTING JAR. A. Glass pressure tube just penetrating the cover of the jar and connected with the atomizer bulbs by rubber tubing. B. glass delivery tube passing through the cover and extending nearly to the bottom of the jar. To this is attached the cannula. C. Injecting cannula, terminating the delivery tube. D. Rubber tubing serving to make the joints air tight where the tubes penetrate the cover of the jar.

1. INJECTING JAR. The preparation of the injecting jar here figured grew out of the necessity of some simple and efficient apparatus for injecting liquids (chloride of gold, nitrate of silver, nitric, chromic, osmic and picric acids) which would be injured by or injure an ordinary syringe. As will be seen by a glance at the figure, the injecting jar is made on the principle of an ordinary wash bottle. It is prepared by boring two holes in the glass cover of a fruit jar or of an anatomical specimen jar, and inserting glass

tubes, the pressure tube just penetrating the cover and the delivery tube extending nearly to the bottom of the jar; (Fig. 1) where the glass tubes penetrate the cover they are surrounded by rubber tubing to render the joints air tight. The pressure is obtained by the use of an atomizer bulb, or, in order that it may be constant, two bulbs are used, the second one being covered with a net to prevent undue distention. The bulbs used with ether-freezing microtomes or with some form of thermo-cautery are good, but two thick walled atomizer bulbs are just as efficient.

The delivery tube and the cannula are of glass, only enough rubber tubing being used to make the delivery tube outside the jar flexible. While this jar was designed for special liquids, it has been found excellent for making fine injections with gelatin mass. With two bulbs, as in the figure, a pressure of 40 mm. of mercury may be obtained; this is sufficient for most purposes. While water or mercury might be used to obtain the pressure as in the various forms of constant pressure apparatus, the atomizer bulbs are preferred, as it is easier for the operator to control the pressure and adapt it to the individual cases.

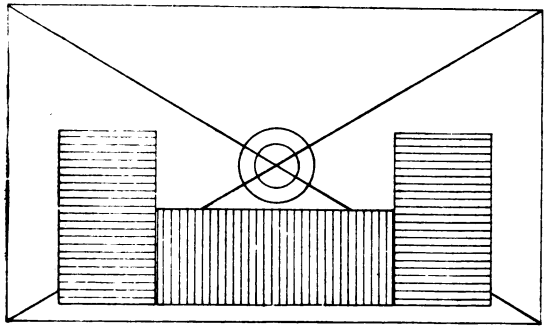

FIG. 2. CENTERING CARD.

2. CENTERING CARD. In mounting objects in balsam or in glycerin without a cell it is somewhat difficult to get the specimen in the center of the slide. It is very easy, however, if the slide is placed upon a card like that shown in the figure (fig. 2). The card is prepared by making upon it several concentric circles, and then cementing to it pieces of glass or Bristol board, so that when the slide is placed in position the center will be over the center of the circles. This device does not enable one to increase the excellence of a preparation, but it does enable one to render the

specimen more attractive to the naked eye and that too without expenditure of extra time.

3. Permanent Caustic Potash Preparations. It is usually stated that specimens treated with caustic potash cannot be permanently preserved. During the past summer an aqueous solution of caustic potash of thirty-five to forty per cent. was used for isolating cardiac muscle from many different animals; as some of the preparations were drawn it seemed unfortunate not to be able to render them permanent as vouchers for the drawings. This was accomplished by adding glacial acetic acid to the isolated cells. The acid combines with the caustic potash to form acetate of potash, which is often used for permanent mounting; finally a mixture of glycerin seventy-five parts and an aqueous solution of piero-carmine (one per cent.) was added as a permanent mounting medium. These specimens after three months show no signs of deterioration. If the specimens were already under the cover-glass, a drop of glacial acetic acid was drawn under it and afterward a drop of the glycerin and picrocarmine mixture.

4. Paper for Cleaning the Lenses of Objectives and Oculars. For the last two years the so-called Japanese filter paper (the bibulous paper often used by dentists when filling teeth) has been used in the laboratory for cleaning the lenses of oculars and objectives, and especially for removing the fluid used with immersion objectives. Whenever a piece is used once it is thrown away. It has proved more satisfactory than cloth or chamois, because dust and sand are not present; and from its bibulous character it is very efficient in removing liquid or semi-liquid substances. At the writer's suggestion it was tried in the Bureau of Animal Industry at Washington, and is now used there almost exclusively for the purpose named above.

5. Demonstration of the Fibrillae of Unstriated Muscular Fibers. For demonstrating the longitudinal fibrillation of unstriated muscular fibers the following method has proved very satisfactory: Ten to fifteen cm. of perfectly fresh small intestine from a cat or other animal is tied at one end and into the other is injected (with the injecting jar, see fig. 1.) the following mixture. ninety-five per cent. alcohol, twenty-five cc., water seventy-five cc: picric acid crystals three-fourths of a gram. When the intestine is

moderately distended the end in which the injection is made is tied and the piece of intestine placed in a glass dish and covered with the mixture. After one or two days the muscular coats may be torn off in shreds. If one of the shreds is teased well with needles, unstriated muscular fibers may be partly or wholly isolated. They may be mounted in seventy-five per cent. glycerin. The picric acid stains the fibers yellow and with a homogeneous immersion (1-12th or 1-18th) the longitudinal fibrillation shows with the greatest clearness. In some cases the ends of the fibers will be frayed and show the fibrillae something like a brush.

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November 1, 1886.